was heated for 2–4 hours at 100°. Any excess of unused amine and solvent was removed by evaporation *in vacuo*. The residue was basified with a small amount of cold 20% aqueous sodium hydroxide (to *p*H 11) and the precipitated solid bis-aminoamide was collected by suction and washed with a little cold water. The products were purified by recrystallization from ethyl acetate, hexane or mixtures of these solvents. Yields were usually above 90%.

The bis-tertiary-aminoacetylamides were quaternized by refluxing with methyl iodide in methanol for several hours. The bis-methiodides crystallized from methanol or methanol–ethyl acetate mixtures. Yields were all above 90%.

Details for all compounds appear in Table I.

TUCKAHOE 7, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF THE SCHERING CORPORATION]

Antifungal Agents[1]

BY STEPHEN B. COAN AND DOMINICK PAPA

RECEIVED OCTOBER 28, 1954

A series of tertiary aliphatic and heterocyclic amines have been prepared and examined as antifungal agents for pathogenic molds and fungi. Certain structure–activity relationships are evident and are discussed.

With the advent of broad-spectrum antibiotics which somewhat indiscriminately destroy both malevolent and benevolent bacteria within the human host, the development of pathogenic fungal infections has received considerable attention.[2] Although there is apparently no completely satisfactory method for combatting fungal infections arising from *M. albicans* and *T. mentagrophytes*, whose overgrowth may be made possible by the destruction of competitive flora, the true incidence of the condition, specifically moniliasis, is still questionable.[2] Despite considerable work and the evolution of several theories, the mechanism whereby *M. albicans* acquires pathogenesis following antibiotic therapy is still obscure.

Despite the lack of agreement among clinicians regarding the need for antifungal agents specifically effective against pathogenic fungi, various types of fungicides are in clinical use or under investigation. Being cognizant of the anti-monilial properties of a group of steroidal amines and their quaternary salts[3] as well as the fungistatic and fungicidal action of certain long chain saturated and unsaturated fatty acids,[4] it was of interest to prepare and examine microbiologically a series of saturated and unsaturated tertiary amines and salts thereof.

The compounds prepared in this study are of the general formula $R_1NR_2R_3$ wherein R_1 is a saturated or unsaturated hydrocarbon group and $—NR_2R_3$ is a dialkylamino, pyrrolidino, piperidino and morpholino group. It is to be noted that these compounds combine the hydrocarbon moiety of the fatty acids[4] and the basic moiety of the steroidal amines.[3]

The tertiary amines were prepared by conventional methods, the procedure of choice being the reaction of the appropriate acid chloride and secondary amine to the intermediate amide (see Table I) followed by reduction of the amide with lithium aluminum hydride.[5]

The amides, tertiary amines and the acid addition and quaternary salts of the latter were submitted to an *in vitro* microbiological test to determine their activity against *Monilia albicans*, *T. mentagrophytes* (Table II) as well as *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *M. smegmatis*. The antifungal activities of the compounds were compared with two fungicides in clinical use, undecylenic acid and 6-(β-diethylaminoethoxy)-2-dimethylaminobenzothiazole dihydrochloride.[6] In order to obtain sufficient data for a structure correlation several known tertiary amines were prepared and examined.

All the compounds were essentially inactive against *E. coli* and *P. aeruginosa*. The amides exhibited activities which were neither of sufficient magnitude nor broadness of spectrum as to warrant reporting. From Table II certain relationships between structure and activity are manifest. It is of interest to note that within the confines of the optimum structures all compounds possessed antifungal activities greater than that of undecylenic acid and in most cases in excess of that possessed by Asterol.

Optimum activity resides in those compounds wherein R_1 = 10-undecenyl, and R_2 and R_3 contain a total of 4–6 carbon atoms (X, XIII, XIV, XV and XVI). Increasing the carbon chain in R_2 and R_3 to isobutyl (XI) or shortening to methyl (IX) markedly reduced activity.

Hydrogenation of the terminal double bond of compound X did not radically alter the antifungal activity (XIX) whereas transforming the ethylenic bond to an acetylenic link narrows the spectrum (XII). The pronounced activity of XIX was of interest since this investigation was originally based upon the supposition that the terminal unsaturation was a prerequisite for activity. Wyss, *et al.*,[4] demonstrated the rather singular properties of undecylenic acid as a fungicide.

Having somewhat empirically shown the effect

(1) Presented in abstract at The North Jersey Miniature Meeting in Newark, New Jersey, January 24, 1955.

(2) (a) M. J. Lipnik, A. M. Kligman and R. Strauss, *J. Investigative Dermat.*, **18**, 247 (1952); (b) W. I. Metzger, L. T. Wright and J. C. DiLorenzo, *J. Am. Med. Assoc.*, **155**, 352 (1954).

(3) (a) F. C. Kull, G. A. Castellano and R. L. Mayer, *J. Investigative Dermat.*, **21**, 227 (1953); (b) Hershel L. Herzog, Constance C. Payne and E. B. Hershberg, to be published.

(4) (a) O. Wyss, B. J. Ludwig and R. R. Joiner, *Arch. Biochem.*, **7**, 415 (1945); (b) J. Kimmig and H. Rieth, *Arzneim. Forsch.*, **3**, 267 (1953).

(5) The reduction (and isolation) was essentially that described by V. Micovic and M. Mihailovic, *J. Org. Chem.*, **18**, 1190 (1953).

(6) Asterol—Registered trade mark of Hoffmann-LaRoche, Inc.

of varying R_2 and R_3, the optimum length of R_1 was determined. From Table II it is evident that shortening the chain to eight carbon atoms (XVII) results in pronounced loss of activity. Optimum activity with $R_2 = R_3$ = ethyl appears to reside in compounds wherein R_1 has 10–12 carbon atoms with slight reduction of activity with a 14 carbon chain. Preliminary studies indicate that with an 18 carbon chain the activity and spectrum is markedly reduced.

In summary it may be stated that among the aliphatic tertiary amines, fungicidal activity varies with chain length, and the optimum *in vitro* activity lies in that group of compounds

TABLE I

AMIDES, $RCONR_2R_3$

Compound no.	R_1	$-NR_2R_3$	Yield, %	B.p. °C.	Mm.	n_D	°C.	Analyses, % Nitrogen Calcd.	Found
I	$C_{10}H_{19}$[a]	Dimethylamino	82.5	115–122	1–2	1.4635	28	6.63	6.43
II	$C_{10}H_{19}$	Diethylamino	80.0	145–150	2	1.4629	23	5.83	5.70
III	$C_{10}H_{19}$	Di-*i*-butylamino[b]	61.0	134–137	2	1.4593	28	4.74	4.55
IV	$C_{10}H_{17}$[c]	Diethylamino	78.2	164–167	2	1.4686	25	5.89	5.58
V	$C_{10}H_{19}$	Pyrrolidino	76.5	167–170	3–4	1.4818	23	5.90	5.65
VI	$C_{10}H_{19}$	Piperidino	74.5	159–162	2	1.4821	22	5.57	5.02
VII	$C_{10}H_{19}$	β-Pipecolino	51.2	160–163	2–3	1.4790	23	5.28	5.06
VIII	$C_{10}H_{19}$	Morpholino	82.0	158–162	1	1.4817	27	5.53	5.31

[a] 10-Decenyl. [b] Calcd. for $C_{19}H_{37}NO$: C, 77.22; H, 12.62. Found: C, 77.51; H, 12.47. [c] 10-Decynyl.

TABLE II

TERTIARY AMINES AND ANTIFUNGAL ACTIVITY, $R_1NR_2R_3 \cdot X$

Compound no.	R_1	$-NR_2R_3$	X	Yield, %	B.p., °C. (mm.) or m.p., °C.	$n^{24}D$	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Minimum concn. prohibiting growth of *Monilia albicans*	Minimum concn. prohibiting growth of *T. mentagrophytes*
IX	$C_{11}H_{21}$[a]	Dimethylamino	....	59.2	89–91(2)	1.4416	...	...	...	...	7.09	6.84	1:40,000	1:100,000
X	$C_{11}H_{21}$	Diethylamino	....	66.2	136–138(11)	1.4473	...	...	...	...	6.21	5.92	1:100,000	1:100,000
X	$C_{11}H_{21}$	Diethylamino	Maleate	74.5	34.0–35.0		66.82	66.48	10.33	10.31	4.10	4.04	1:40,000	1:100,000
X	$C_{11}H_{21}$	Diethylamino	CH_3I	50.0	89.0–90.0		53.39	52.98	9.33	9.50	3.84	3.61	>1:5,000	
XI	$C_{11}H_{21}$	Di-*i*-butylamino	....	57.0	133–140(2)	1.4464	81.08	81.24	13.96	14.00	4.97	4.80	>1:5,000	>1:50,000
XII	$C_{11}H_{19}$[b]	Diethylamino	....	70.0	112–115(3)	1.4506	...	...	...	...	6.23	6.69	1:10,000	1:100,000
XIII	$C_{11}H_{21}$	Pyrrolidino	....	65.5	153–156(10)	1.4644	...	...	...	...	6.27	5.94	1:100,000	1:200,000
XIII	$C_{11}H_{21}$	Pyrrolidino	Maleate	33.0	52.5–53.0		67.21	67.53	9.79	9.55	4.13	3.90	1:40,000	1:100,000
XIV	$C_{11}H_{21}$	Piperidino	....	65.0	160–164(10)	1.4667	...	...	...	...	5.90	5.62		
XIV	$C_{11}H_{21}$	Piperidino	Maleate	76.5	77.5–78.0		67.95	68.30	9.98	9.73	3.96	3.48	1:80,000	1:100,000
XV	$C_{11}H_{21}$	β-Pipecolino	....	68.2	156–159(8)	1.4633	...	...	...	...	5.57	5.40	1:100,000	1:120,000
XV	$C_{11}H_{21}$	β-Pipecolino	Maleate	73.5	83.0–83.5		68.66	68.60	10.15	9.92	3.81	3.30	1:80,000	1:200,000
XV	$C_{11}H_{21}$	β-Pipecolino	CH_3I	52.0	132.8–133.0		54.95	54.73	9.22	8.96	3.56	3.01	>1:5,000	
XVI	$C_{11}H_{21}$	Morpholino	....	53.5	123–129(2)	1.4638	...	...	...	...	5.60	5.40	1:40,000	1:100,000
XVII	C_8H_{17}[c]	Diethylamino	....	36.0	105–106(17)[d]	1.4320	...	...	...	...	..	..	1:5,000	>1:50,000
XVIII	$C_{10}H_{21}$[e]	Diethylamino	....	80.0	75–80(1)	1.4362	...	...	...	...	6.57	6.74	1:100,000	1:400,000
XVIII	$C_{10}H_{21}$	Diethylamino	Hydrochloride	76.7	116.8–117.0		67.29	67.10	12.91	13.00	5.67	5.88	1:25,000	1:800,000
XIX	$C_{11}H_{23}$[f]	Diethylamino	....	90.0	107–111(3)[g]	1.4382	...	...	...	...	6.16	6.20	1:40,000	1:400,000
XX	$C_{12}H_{25}$[h]	Diethylamino	....	76.5	120–125(3)[i]	1.4410	...	...	...	...	..	..	1:100,000	1:1,600,000
XX	$C_{12}H_{25}$	Diethylamino	Hydrochloride	72.0	115.0–115.5[i]		...	...	...	...	..	..	1:50,000	1:800,000
XXI	$C_{14}H_{29}$[j]	Diethylamino	Hydrochloride	38.0	119.0–120		71.59	71.38	12.08	11.82	4.64	5.17	1:50,000	1:800,000
	Undecylenic acid												1:10,000	1:10,000
	Asterol												1:40,000	1:50,000

[a] 10-Undecenyl. [b] 10-Undecynyl. [c] *n*-Octyl. [d] Reported 98° (13 mm.), by J. Von Braun and K. Weissbach, *Ber.*, **63B**, 489 (1930). [e] Decyl. [f] Undecyl. [g] Reported 85–88°, (2 mm.), by D. A. Peak and T. I. Watkins, *J. Chem. Soc.*, 3292 (1951). [h] Dodecyl. [i] Reported 122–124° (2 mm.), $n^{19}D$ 1.443, HCl: m.p. 119.5° by O. Westphall and D. Yerchel, *Ber.*, **73**, 1002 (1940). [j] Tetradecyl.

wherein R_1 contains 10–14 carbon atoms in a chain and R_2 and R_3 together contain approximately 4–6 carbon atoms.

Acknowledgment.—The authors wish to express their appreciation to Mr. E. Foley, Miss Marilyn Bronit, Mr. J. Stevenson and Mr. F. Vitale of our Bacteriology Department for the microbiological data herein reported. The authors also express their appreciation to Mr. Edwin Conner for the microanalyses.

Experimental

Preparation of Amides.—The following procedure is typical of that used in the preparation of the amides utilizing the appropriate acid chloride and secondary amines.

To a solution of 56.8 g. (0.8 mole) of pyrrolidine in 300 ml. of anhydrous benzene was added dropwise 80.8 g. (0.4 mole) of undecenoyl chloride in 200 ml. of anhydrous benzene. The mixture was stirred and refluxed for 1–2 hours, cooled, poured into water and extracted with dilute hydrochloric acid, sodium bicarbonate solution and water in turn. After drying over anhydrous sodium sulfate, the solvent was removed *in vacuo* and the residue distilled yielding 73 g. (0.32 mole, 75%) of ω-undecenoylpyrrolidine, b.p. 167–170° (3–4 mm.).

Preparation of Amines, Reduction of Amides.—The following procedure is typical of that employing the appropriate amide as starting material and is modelled after the procedure of Micovic and Mihailovic.[5]

To a stirred suspension of 7.1 g. (0.144 mole + 30% excess) of lithium aluminum hydride in 500 ml. of anhydrous ether was added dropwise 0.287 mole of the appropriate amide in 200 ml. of anhydrous ether. The mixture was stirred and refluxed for two hours after which time the reaction vessel was cooled in an ice-bath. The reaction mixture was decomposed by the cautious dropwise addition in turn of 7 ml. of water, 7 ml. of 15% sodium hydroxide solution and 21 ml. of water while stirring vigorously during the addition and for 20 minutes afterwards. The mixture was filtered and the precipitated salts washed with ether. The combined ether filtrates were extracted with 10% hydrochloric acid. The acid layer was rendered alkaline with sodium hydroxide and extracted with ether. The ether solution was dried over anhydrous potassium carbonate, concentrated *in vacuo* and distilled *in vacuo* to yield the desired amine.

Alkylation of Secondary Amines.—A mixture of 0.75 mole of secondary amine, 0.3 mole of alkyl bromide, 100 ml. of anhydrous toluene and 50 ml. of anhydrous ethanol was refluxed overnight whereupon it was cooled, poured into water and extracted with ether–benzene, 1:1. The organic layer was extracted with dilute hydrochloric acid and the acid layer was made strongly basic with sodium hydroxide solution. The aqueous mixture was extracted with ether–benzene and the extracts dried over anhydrous potassium carbonate. Removal of the solvent *in vacuo* followed by fractionation of the residue yielded the desired tertiary amine.

Reduction of an Unsaturated Tertiary Amine. Undecyldiethylamine (XIX).—A solution of 22.5 g. (0.1 mole) of ω-undecenyldiethylamine in 150 ml. of ethanol was hydrogenated in a Parr shaker under 3 atmospheres of hydrogen in the presence of platinum oxide catalyst. When the reduction was complete, the catalyst was removed by filtration, the solvent concentrated *in vacuo* and the residue distilled to yield *undecyldiethylamine*.

Alkylation of a Primary Amine. Tetradecyldiethylamine Hydrochloride (XXI).—A mixture of 42.6 g. (0.2 mole) of tetradecylamine, 65 g. (0.42 mole) of ethyl iodide, 25 g. (0.25 mole) of potassium carbonate and 500 ml. of anhydrous benzene was stirred and refluxed for 48 hours. The mixture was then poured into water and extracted with ether–benzene 1:1. The organic layer was dried over anhydrous potassium carbonate, concentrated and distilled *in vacuo*, collecting 29 g., b.p. 160–190° (5 mm.). The mixture of amines was refluxed with 24 g. of acetic anhydride and 50 ml. of glacial acetic acid for 6 hours. The reaction mixture was then cooled, poured into water and rendered alkaline with sodium hydroxide solution. The mixture was extracted with ether which was then extracted with dilute hydrochloric acid. The acid layer was made alkaline and extracted with ether. The ether solution was dried over anhydrous potassium carbonate and filtered. Anhydrous hydrogen chloride was bubbled through the ether solution and the precipitated hydrochloride removed by filtration and recrystallized from ethyl acetate yielding 20 g., m.p. 119.0–120.0°.

Preparation of Salts. A. Maleates.—Equimolar quantities of tertiary amine and maleic acid in 4–5 volumes of isopropyl acetate were heated for a few moments and the resultant solution concentrated to a residue *in vacuo*. The glassy residue was recrystallized from anhydrous ether and the salt was obtained as a somewhat hygroscopic waxy solid.

B. Hydrochlorides.—A dilute ether solution of the tertiary amine was treated with anhydrous hydrogen chloride and the precipitated salt recrystallized from ethyl acetate.

C. Methyl Iodides.—A mixture of the tertiary amine and excess methyl iodide in anhydrous ether was allowed to stand overnight. The soft crystals of the quaternary salt were removed by filtration and recrystallized from ethyl acetate.

Method of Testing.—Growth inhibition activity was determined by culturing agar plates containing twofold dilutions of the compounds to be tested. Bacteria and Monilia cultures grown in broth for 24 hours were streaked on tryptose agar (Difco) containing 5% serum (*p*H 7.2). The cultures were examined for growth after 48 hours incubation at 37°. Tryptose agar plates (without serum added) were streaked with heavy spore suspensions of *T. mentagraphytes*, and were examined for growth after two weeks incubation at room temperature.